ABSTRACT

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Title:

Effect of storage of biological material on activity of biotransformation enzymes

Effect of storage of liver and subcellullar fractions on the activities of biotransformation enzymes was studied. Activities of oxidation enzymes – cytochromes P450 (CYP1A, 2B, 2C, 3A) and flavin-containg monooxygenases (FMO), carbonyl-reducing enzymes (AKR1A1, AKR1C, 11β-HSD,CBR) and conjugation enzymes (UGT, GST) were observed.

Microsomal and cytosolic subcellular fractions prepared from differently stored liver of sheep (*Ovis aries*) were used. (A) from fresh tissue frozen in the liquid nitrogen. (B) from liver that was stored at -80°C, the temperature gradually increased to 0°C and then gradually decreased back to -80°C. The half of prepared microsomal and cytosolic subcellular fractions from liver stored in liquid nitrogen (A) was processed by the same storage procedure as liver (B). The second half was stored at -80°C.

Activities of tested biotransformation enzymes were determined toward their relatively specific substrates. The subcellular fractions were also incubated with xenobiotics (oracin, flubendazole and albendazole) and the concentrations of known metabolites of these drugs were quantified by HPLC. Obtained results were statistically evaluated.

Thawing and re-freezing to -80°C of sheep liver tissue before preparing samples depressed activity of microsomal CYP1A, UGT, 11β-HSD 1 (toward oracin) a CYP3A (toward ABZ), CBRs (toward FLU) and of cytosolic GST. On the other hand activity of FMO (toward TB) was increased. This procedure also causes rising of protein concentration in both subcellular fractions.

Thawing and re-freezing of prepared subcellular fractions caused decrease of activity of AKR1C (toward acenaphthenol) and GST in cytosole, 3α-HSD (toward 4-pyridincarboxaldehyde), CYP1A1, 1A2, 2B, 2C9, 3A, 11β-HSD 1 (toward oracin), CYP 1A (toward ABZSO) in microsomes.

The oxidative enzymes seem to be the most affected by the way of storage of biological samples. Activity of conjugation enzymes was decreased in both cases. Carbonyl-reducing enzymes seem to be least sensitive to the process of storage of biological material.